

## Analysis of Peptide-Protein and Monoclonal Antibody with UHPLC II

#### 1. Column for Macromolecule

Proteins and monoclonal antibodies (mAbs) have molecular weights of tens to hundreds of kilodaltons. In order to fit the requirements of your analysis for these compounds, the FlexFire WP (Wide Pore) series have a lineup of four types at 300 Å and one monoclonal antibody dedicated column (1000 Å), for a total of five types. This wide range of options allows users to select the best column to fit their needs.

Here we introduce the usefulness of wide pore columns, targeting even lager macromolecules than in the previous report "Monoclonal Antibody Analysis".

### 2. FlexFire WP Series Specifications

	FlexFire mAb-RP	FlexFire WP C4	FlexFire WP C18	FlexFire WP C8	FlexFire WP C1
Particle size	2.6 μm, 5 μm				
Chemistry	Butyl	Butyl	Octadecyl	Octyl	Trimethyl
Surface area	24 m²/g	170 m²/g	170 m²/g	170 m²/g	170 m²/g
Pore Volume	1.4 mL/g				
Pore diameter	100 nm	30 nm	30 nm	30 nm	30 nm
Carbon	1.3%	5%	15%	7%	3%
End-cap	0	0	0	0	0
рН	pH1-10	pH1-10	pH1-10	pH1-10	pH1-10
Temperature	~80°C	~80°C	~80°C	~80°C	~80°C
	2.6 µm: 600 bar (=60Mpa=8,702 psi)				
	5 µm: 300 bar (=30Mpa=4,351 psi)				



#### 3. Recovery Rate of mAbs

The figure and table show the recovery rate of mAbs(M.W.150K) by each column. Columns with alkyl chains of C4 or less provide better linearity and higher recovery rate.

Column	R <sup>2</sup>	Recovery (%)
FlexFire WP C1	0.99886	104
FlexFire WP C4	0.99961	103
FlexFire WP C8	0.99795	89.8
FlexFire WP C18	0.99823	98.1
FlexFire mAb-RP	0.99886	101



### 4. Comparison of Separation of Myoglobin and Unknown Compounds



#### Conditions; Column: FlexFire WP Series, 2.6µm (2.0x50mm) Mobile phase: A) Water+0.1%TFA B) Acetonitrile+0.1%TFA Gradient: mL/min %A %B Curve min 0.00 0.3 80 20 8.40 0.3 40 60 6 8.42 0.3 80 20 6 40℃ Temperature: UV280nm Detection: Sample: Myoglobin Injection volume: 0.2µL System: Waters ACQUITY UPLC H-Class PLUS Mixer: 100µL

# Separation of myoglobin and unknown compounds

All columns give good results of separation of myoglobin and unknown compounds. Since a high degree of separation is obtained with FlexFire mAb-RP, WP C8, and WP C4, it is effective in separating multiple components.



#### 5. Analysis of Protein



### Analysis of Lactalbumin

Compared to a column with pore size 120Å, those with pore size 300Å and 1000Å presented better peak shapes. Especially the column with pore size 1000Å achieved clearer peak separation.





7



#### Conditions:

Gradient:	min	mL/min	%A	%B	Curve	
	0.00	0.3	80	20		
	8.40	0.3	40	60	6	
	8.42	0.3	80	20	6	
Temperature:	40°C					
Detection:	UV210r	m				
Sample:	1.Ribonuclease A (13.7KDa)					
Sample:	1.Ribon	uclease A (	13.7KDa	)		
Sample:	1.Ribon 2.Cytoc	uclease A ( hrome C (1	13.7KDa 2.4KDa)	)		
Sample:	1.Ribon 2.Cytoc 3.Lysoz	uclease A ( hrome C (1 yme (14.3k	13.7KDa 2.4KDa) (Da)	)		
Sample:	1.Ribon 2.Cytoc 3.Lysoz 4.BSA (	uclease A ( hrome C (1 yme (14.3k 66.3KDa)	13.7KDa 2.4KDa) (Da)	)		
Sample:	1.Ribon 2.Cytoc 3.Lysoz 4.BSA ( 5.Myogl	uclease A ( hrome C (1 yme (14.3k 66.3KDa) obin (11.2k	13.7KDa 2.4KDa) (Da) (Da)	•)		
Sample:	1.Ribon 2.Cytoc 3.Lysoz 4.BSA ( 5.Myogl 6.Catala	uclease A ( hrome C (1 yme (14.3k 66.3KDa) obin (11.2k ase (220KD	13.7KDa 2.4KDa) (Da) (Da) a)	)		
Sample: Injection volum	1.Ribon 2.Cytoc 3.Lysoz 4.BSA ( 5.Myogl 6.Catala e: 2.0µL	uclease A ( hrome C (1 yme (14.3k 66.3KDa) obin (11.2k ase (220KD	13.7KDa 2.4KDa) (Da) (Da) a)	)		
Sample: Injection volum System:	1.Ribon 2.Cytoc 3.Lysoz 4.BSA ( 5.Myogl 6.Catala e: 2.0µL Waters	uclease A ( hrome C (1 yme (14.3k 66.3KDa) obin (11.2k ase (220KD ACQUITY U	13.7KDa 2.4KDa) (Da) (Da) a)	a)	s	

## Comparison of separation between myoglobin and catalase

Even though it is possible for a column with a pore size of 300Å to separate at MW 10kDA-60kDA, for macromolecules with MWs of 200 kDA larger pore sizes such as 1000Å and 450Å achieved better separation.

By using FlexFire mAb-RP, it can be expected not only to separate between macromolecules but also to detect unknown compounds contained in protein samples.



#### 7. **Monoclonal Antibody Analysis**



Gradient:	min	mL/min	%A	%B	Curve
	0.00	0.3	80	20	
	8.40	0.3	40	60	6
	8.42	0.3	80	20	6
Detection: Sample: Injection volume:	40°C UV280nr NISTmAl 1.0µL	n b			
System:	Waters A	ACQUITY UP	LC H-Clas	s PLUS	
Mixer:	100µL				





#### Monoclonal antibody analysis example

In the previous technical report "Antibody Analysis with UHPLC-MS", we focused on using columns with a 300Å pore size. In this report, we found columns with a pore size of 1000Å produce sharper peaks and enabled us to gain new insights.

You can find analysis examples of a column with pore size 300Å in the technical report "Antibody Analysis with UHPLC-MS".

colorini.	TIEXTITE	1100 117, 210	pin (2.0)	(Southing)	
Mobile phase:	A) Water	r + 0.196TFA	B) Ace	tonitrile -	- 0.196TF
Gradient:	min	mL/min	96A	96B	Curve
	0.00	0.3	80	20	
	8.40	0.3	40	60	6
	8.42	0.3	80	20	6
Temperature:	40°C				
Detection:	UV280nr	n			
Sample:	NISTMA	b, Reduced I	VISTmAb		
Injection volume:	1.0µL				
System:	Waters A	CQUITY UP	LC H-Clas	SS PLUS	
Miscore	10001				

#### Analysis of mAb reductant

The reductant was made by mixing 20 mM DTT with NISTmAb in 1: 1, and the reaction was carried out at 40 °C-2 hrs.

> You can find analysis examples of a column with pore size 300Å in the technical report "Antibody Analysis with UHPLC-MS".



#### 8. **Peptide Mapping**



Conditions;							
Column:	FlexFire	C18, 2.6µm ()	2.0x50m	nm)			
	FlexFire	C18, 1.6µm (	2.0x50m	m)			
	FlexFire	WP C18, 2.6u	m (2.0x	50mm)			
	ElexFire WP C18, 1.6um (2.0x50mm)						
	FlexFire	WP C4, 2.6un	n (2.0x5	(mm)			
	FlexFire	mAb-RP, 2.6µ	m (2.0	(50mm)			
Mobile phase:	A) Wate	r + 0.1%TFA	B) Ace	tonitrile +	+ 0.196TFA		
Gradient:	min	mL/min	96A	96B	Curve		
	0.00	0.3	80	20			
	8.40	0.3	40	60	6		
	8.42	0.3	80	20	6		
Temperature:	40°C						
Detection:	UV280nr	n					
Sample:	BSA Dige	est					
Injection volume:	1.0µL						
System:	Waters /	ACQUITY UPL	C H-Clas	ss PLUS			
Mixer:	100µL						

"Thermo Scientific TM SMART Digest TM Kit (Thermo Fisher Scientific)" was used for protein digestion. If your samples include only digested peptides, a standard column with a pore size of 120Å can be an option. However, you can get better results if you match the pore size of the column to the molecular weight

#### 8-1. mAb Tryptic Digest



e phase:	A) Water	r + 0.1%	A B) ACE	tonitrile -	0.1961
ent:	min	mL/min	96A	%B	Curve
	0.00	0.3	80	20	
	8.40	0.3	40	60	6
	8.42	0.3	80	20	6
erature: tion: le: ion volume:	80°C UV280nr NISTmAl 1.0µL	n b Digest			
m:	Waters A	CQUITY UP	LC H-Clas	S PLUS	

Monoclonal antibodies frequently have molecular weights near 150 kDa. In "7. Monoclonal antibody analysis", the utility of using mAbs 1000Å pore size for intact mAbs was revealed. You can use the same column for both intact analysis and digest analysis in peptide mapping.



#### 8-2. Tryptic Digest of Hemoglobin



Conditions; Column: Mobile phase:

FlexFire mAb-RP, 2.6µm (2.0x50mm) A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

0.00	0.3	00		
	0.0	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6
	8.40 8.42	8.40 0.3 8.42 0.3	8.40 0.3 40 8.42 0.3 80	8.40 0.3 40 60 8.42 0.3 80 20

Detection: Sample: Injection volume: System:

#### 1.0µL Waters ACQUITY UPLC H-Class PLUS 100µL

Hemoglobin Digest

#### 8-3. Tryptic Digest of ADH



min	mL/min	%A	%B	Curve
 0.00	0.3	80	20	
8.40	0.3	40	60	6
8.42	0.3	80	20	6

ADH Digest 1.0µL

#### Waters ACQUITY UPLC H-Class PLUS 100µL

#### 8-4. Analysis of Synthetic RNA





#### 9. Analysis of Synthetic RNA



#### 10. Analysis of pBR322 Digest





EtBr 染色

100uL Mixer:

The FlexFire WP C18 enables analysis of samples containing macromolecules too large for use with the FlexFire C18 (120Å).



Contact Us



Develosil USA 10060 Carroll Canyon Rd. Ste. 100 San Diego, CA 92131 Phone: 858-800-2433 Web: https://develosil.us/



Nomura Chemical Co., Ltd. 15, Hinode-cho, Set, 489-0004, Japan Tel: +81-561-48-1853 Fax: +81-561-48-1434 E-mail: info@develosil.net

The products specifications may change without notice as products are improved. Thank you in advance for your understanding.

## www.develosil.us